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MUTAGENIC ACTIVITY OF DIMETHYLSULFOXIDE (DMSO) SOLVENT SAMPLES FROM MUNITION PILOT TEST PLANT ON MAMMALIAN CELLS

FINAL REPORT

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Two DMSO solvent samples containing munition constituents contained a direct-acting mutagen(s). Of the two samples, DMSO recycle solvent sample had a more acute mutagenic activity than DMSO evaporator sludge sample at comparable dilution of the stock solution. The mutagenic activity in these samples was not augmented with addition of rat liver S-9. Since these samples are a mixture of several compounds, the identity of the specific compound(s) responsible for the mutagenic activity is not known.

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ABSTRACT

Two DMSO solvent samples containing munition constituents contained a direct-acting mutagen(s). Of the two samples, DMSO recycle solvent sample had a more acute mutagenic activity than DMSO evaporator sludge sample at comparable dilution of the stock solution. The mutagenic activity in these samples was not augmented with addition of rat liver S-9. Since these samples are a mixture of several compounds, the identity of the specific compound(s) responsible for the mutagenic activity is not known.

INTRODUCTION

Epidemiological surveys and experiments on animals have provided data which indicate that man-made and natural chemicals are probably responsible, directly or indirectly, for many of the human cancers. In order to identify these potentially hazardous compounds in the work environment, efforts have been directed toward the development of rapid *in vitro* assays that can differentiate genotoxic from non-genotoxic compounds.

The development of *in vitro* gene mutation assays, using either microorganisms (Ames et al., 1975) or mammalian cells (Hsie et al., 1975; Clive and Spector, 1975), has permitted rapid identification of mutagens and potential carcinogens in the work environment. While the bacterial cells are frequently used for the detection of mutagens/carcinogens, the bacterial genetic structure is much simpler than that of mammalian cells and may not represent the potential susceptibility of mammalian cells to mutation by environmental pollutants. The haploid bacterial genes consist only of functional genes or exons while the diploid mammalian cell genes consist of both functional exons and non-functional introns. Thus, the use of a mammalian cell system for evaluation of mutagens/carcinogens *in vitro* may give a better estimate of the potential hazards of environmental pollutants at the work place. This research project, as part of the Department of Defense program to develop a biomedical and scientific data base on dimethylsulfoxide (DMSO) solvent used in munition process streams, is to provide an occupational health protection criterion for workers involved with the munition production process.

This study evaluates the mutagenicity of munition contaminated DMSO solvent from the pilot plant recrystallization facility using a mammalian cell mutagenicity assay. The data from this study indicate that the two DMSO solvent samples containing munition constituents contain a direct-acting mutagen(s). The mutagenic activity in these samples is not enhanced by the addition of rat liver S-9. Of the two samples tested, DMSO recycle solvent sample had a more acute mutagenic activity than DMSO evaporator sludge at the same dilution of the stock solutions.

METHODS AND RESULTS

The mouse lymphoma cell culture (L5178Y) with phenotype $TK^{+/-}$ which was received from Dr. Clive and frozen in liquid nitrogen was used as the stock cell culture for the present study. The cells were propagated in Fischer's medium supplemented with 10% v/v heat-inactivated horse serum ($F_{10}p$). The mouse lymphoma cell mutagenicity assay was conducted as previously described by Clive and Spector (1975). Briefly, six million cells from an actively growing culture were exposed to various concentrations of known and unknown potential mutagens in 10 ml $F_{10}p$ medium for four hours at 37°C with constant agitation. At the end of four hours, the cells were washed free of the compound and then suspended in $F_{10}p$ medium. The cells were agitated and passed

three times at 24-hour intervals at 3 x 10^5 cells per ml. After the third passage, the control and treated cells were cloned in 0.37% agar in $F_{20}P$ with and without trifluorothymidine (TFT at 1.0 µg/ml). The cells (300 cells per plate for viability test and 1 x 10^6 cells per plate for the mutagenicity test) were suspended in 15 ml of agar medium, gassed with 5% CO_2 in air and then incubated at 37°C. After 10 days clonal cell growth in each petri dish was examined and recorded. As control mutagens, ethyl methanesulfonate (EMS) was used as a direct-acting mutagen while 2-acetylaminofluorene (2-AAF) was used as a promutagen which required rat liver S-9 for metabolic activation. Rat liver S-9 was prepared according to Turner et al. (1984). Six mature Sprague-Dawley rats were injected intraperitoneally with 0.5 ml/rat of inducer solution (1.3 gm Aroclor 1242 and 0.67 gm Aroclor 1254 in 8.0 ml corn oil). Five days post injection, the rats were sacrificed and the livers were removed asceptically. The chilled livers were weighed and then suspended in 0.25 M sterile sucrose solution equal to 3 times the weight of the livers. The livers were thoroughly homogenized and then the homogenate was centrifuged at 9,000 x g for 20 minutes at 4°C. The supernatant was collected and stored in sterile vials at -70°C. The rat liver S-9 was evaluated for metabolic activation of 2-AAF and found to have activity comparable to that reported by Turner et al. (1984). The three samples, virgin DMSO solvent (VDS), DMSO recycle solvent (DRS), and DMSO evaporator sludge (DES), were received from Col. J. Fruin, Toxicology Division, Letterman Army Institute of Research (LAIR), San Francisco, California.

The results are presented in Table 1 and shown in Figure 1. The virgin DMSO sample had insignificant mutagenic activity above 4% v/v and was cytotoxic at 10% v/v. The addition of rat liver S-9 did not enhance the mutagenic activity. The two DMSO solvents containing munition constituents were mutagenic at different concentrations. The DRS which was a saturated solution, based on the formation of a precipitate at room temperature, was not very soluble in tissue culture medium. In order to obtain a homogenous solution, the stock sample was heated to 45-50°C before diluting in tissue culture medium. At concentrations greater than 0.1% v/v in medium, a noticeable quantity of precipitate was detected in the medium. Since the precipitate could not be readily removed by washing cells after exposure to the cells, the precipitate was removed from the medium by centrifugation prior to the cells being exposed to the sample. Under these conditions, DRS was found to induce mutation beginning at 0.75% to 1.5% v/v with marked cytotoxicity at the highest concentration. The addition of rat liver S-9 did not augment the mutagenic activity of DRS.

The DES sample which was more soluble was mutagenic between 1.0% to 5.0% v/v and was cytotoxic above 5%. The addition of 10% v/v rat liver S-9 to all test samples did not augment the mutagenic activity in the samples; in fact, the mutagenicity was reduced in all instances.

Although the concentrations of the mutagen in the two DMSO samples are not known, the mutagenic activity in DRS was more acute at a comparable dilution of the stock solution (Figure 1).

Table 1

MUTAGENIC ACTIVITY OF DIMETHYLSULFOXIDE (DMSO) SOLVENT SAMPLES FROM MUNITION PLANTS ON MOUSE LYMPHOMA CELLS

Concentration % v/v[1]	Without S-9 # Mutants/ 10^5 Cells[2]	Without S-9 Cell Survival % of Control	With S-9 # Mutants/ 10^6 Cells[2]	With S-9 Cell Survival % of Control
VDS[3]				
1	0	100	0	100
2	0	100	0	76
4	15	100	6.0	68
6	6.4	95	0	71
8	8.1	83	0	59
10	Toxic	-		
DRS[4]				
0.1	0	100	1	100
0.25	2	100		
.50	3	100	7	100
.75	49	70		
1.00	155	58	12	98
1.25	288	31	0	81
1.50	246	18	15.0	90
2.00	Toxic			
DES[5]				
0.1	0	100	0	100
0.5	0	100	8	100
1.0	8	100	0	100
2.0	102	97	2	100
4.0	321	59	15	88
5.0	212	42	29	89
2.5 mM EMS	192			
50 µg/ml 2-AAF			154	

(1)Dilution of original stock solution

(2)Values corrected for cloning efficiency (65-70% of viable cells) and spontaneous background mutagens (20 mutants/10^6 cells)

(3)Virgin DMSO solvent (VDS)

(4)DMSO recycle solvent (DRS)

(5)DMSO evaporator sludge (DES)

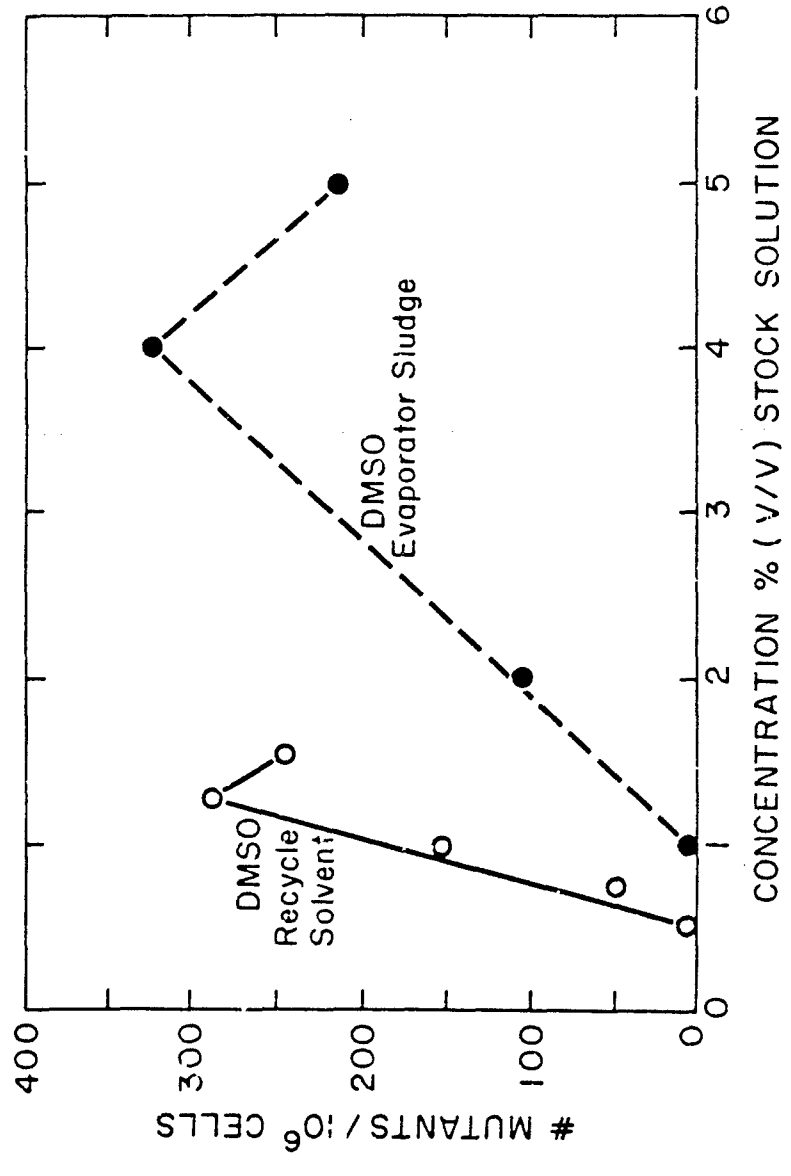

Fig. 1. Mutagenicity in two DMSO solvent samples on mouse lymphoma cells.

DISCUSSION

The present data clearly indicates that both DMSO samples containing munition constituents contain a direct-acting mutagen(s) and the mutagenic activity is not enhanced with rat liver S-9 metabolic activation. These results are partially in agreement with those reported at LAIR (Sauer et al., 1983). They detected mutagenic activity only in DES and not in DRS with the Ames bacterial mutagenicity assay. The reason the assays conducted at LAIR failed to detect mutagens in DRS is not evident at this time. However, if one compares the data obtained at the two laboratories for DES, the results from LEHR, based on the number of mutants, suggest that the mammalian cell assay may be more sensitive to the mutagen(s) in DES. Whether this difference can be attributed to relative sensitivity of the two assays cannot be determined without further studies using concurrently the two assays under identical conditions.

The identity of the mutagen in the two DMSO samples is not known. Initially, based on the assumption that the DMSO samples contained only three compounds, e.g. HMX, RDX, and TAX (Rothrock, 1982), our results suggested that TAX was probably the major mutagen in these samples. However, recent data from LAIR using purified TAX indicated that the substance was not mutagenic in the Ames assay. In addition, they found that the DES and DRS contained more than three components reported earlier (Dr. Dacre, personal communication). Thus, the identity of the specific mutagen in the DMSO samples remain unresolved.

The mutagenic potency of the compound(s) in the DMSO samples cannot be determined without knowing the concentration of the specific components. Since these samples are a mixture of several compounds of unknown concentrations, the mutagenic potency of the mutagen cannot be quantitated. However, if one assumes that the concentration of the mutagen is equal or less than the other compound in aqueous solution, the mutagenic potency of the mutagen in DES is probably greater than EMS, the known mutagen used as a positive control. In addition, the mutagenic potency of the compound in DRS appears to be approximately four-fold greater than the mutagen in DES. Whether this mutagenic potency is from a single compound or an additive or synergistic effect of two or more mutagens is not known. Since EMS is known to be carcinogenic in rodents (IARC, 1974), it is likely that the mutagen(s) in the DMSO samples containing munition constituents could pose a health hazard to those handling these samples. Although the concentration of the specific mutagen(s) is not known and may constitute a minor component in these samples, the potential hazard may come from continuous low-level exposure in the work environment.

CONCLUSION

The conclusion from this study is that the DMSO samples containing munition constituents contain a direct-acting mutagen(s) with a relatively high mutagenic potency when compared with known carcinogens. While the actual identity and mutagenic potency is not known, the data would suggest that the mutagen(s) could pose a health hazard to anyone that is continuously exposed to the DMSO samples. In order to assess the health hazard of the mutagen(s), the DMSO samples should be initially fractionated to identify the specific mutagenic component(s) and then conduct further characterization on the purified compound(s). The purified mutagen(s) should be evaluated for its mutagenic and carcinogenic potential in in vitro and in vivo assays. In addition, since the DMSO samples that will be in the work environment are mixtures of several compounds, reconstituted mixtures that are comparable to the DMSO samples of known pure compounds should be evaluated for a possible additive or synergistic effect on carcinogenesis.

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